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Follow-Up Study of Clinical and Immunological Findings in Patients Presenting With Acute Parvovirus B19 Infection

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This study was undertaken to examine the natural history of parvovirus B19 infection in persons without a known immune defect in terms of both clinical symptoms and immune responsiveness to the virus. Fifty-three patients with acute B19 infection (positive for serum anti-B19 IgM) were studied; symptoms at acute infection were rash and arthralgia (n = 26), rash (n = 7), arthralgia (n = 16), aplastic crisis (n = 3), and intrauterine fetal death (n = 1). Patients were followed for 26–85 months (mean 57 months) and reassessed for persistent symptoms, anti-B19 antibodies, and antibodies to the unique region of B19 VP1. There were 23 cases of arthralgia persisting for longer than 1 year after acute infection. One of these patients, a 48-year-old woman at follow-up, had had persistent arthralgia for 4 years following acute B19 infection, had rheumatoid factor at a titre of 1920 IU/ml detected at follow-up, and had been independently diagnosed as having rheumatoid arthritis at the time of follow-up. All 53 patients were positive for serum anti-B19 IgG compared to 45 of 53 age- and sex-matched control patients, a significant difference (two-tailed *P* value = 0.008). All test patients at follow-up and control patients were negative for serum anti-B19 IgM and antibodies to the unique region of B19 VP1. Serum from acute infection from 33 of 53 test patients was tested for antibodies to the unique region of VP1, and 16 of these were positive. The presence of this antibody did not correlate with subsequent duration of symptoms but did correlate with a short interval between symptom onset and blood sampling. The unique region of B19 VP1 is known to be crucial for a successful humoral response to the virus, and it seems that the antigenic role played by this region is important only during the acute phase of B19 infection.

KEY WORDS: parvovirus B19, persistence, arthralgia, immune response

INTRODUCTION

Human parvovirus B19 is the etiological agent of erythema infectious (El) and transient aplastic crisis (TAC) in patients with red cell aplasia and has been associated with fetal death, acute and chronic arthritis, chronic anaemia in immunocompromised patients, congenital red cell aplasia, and vasculitis syndromes [Brown et al., 1994]. The pattern of B19 disease is strongly influenced by the immune response. Bone marrow depression occurs during the early viraemic phase and under normal circumstances is terminated by a neutralising antibody response. Kurtzman et al. [1989] demonstrated that in humans the early antibody response to B19 infection consists of IgM and is almost entirely VP2-specific. As the response matures, IgG becomes the major antibody subclass and the primary protein detected on immunoblots is VP1, despite its much lower concentration in the virion. VP1 is also the major target specificity of pooled human immunoglobulin [Kurtzman et al., 1989] used in the treatment of persistent infection. In persistently infected patients, including HIV-infected individuals who are able to generate high titres of B19-specific antibody, the switch from predominant IgM and VP2 reactivity to predominant IgG and VP1 reactivity did not occur. Although B19 persistence is most commonly associated with defined immunodeficiency syndromes [Brown et al., 1994], persistent PCR positivity has also been demonstrated in immunocompetent individuals [Faden et al., 1992].

Abnormal immune responses to B19 antigens have also been demonstrated in patients who develop B19 arthropathy. By using unique VP1 peptides as antigen, multiple epitopes were recognised by the serum of individuals with asymptomatic B19 infection (positive for serum anti-B19 IgM); however, patients with self-limiting B19 arthropathy and chronic B19 arthropathy

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lacked these antibodies [Naides et al., 1992]. To examine the natural history of B19 infection in persons without a known immune defect, in terms of both clinical symptoms and immune responsiveness to the virus, we studied 53 patients with B19 infection. These patients, along with controls, were assessed both at acute infection and at follow-up.

MATERIALS AND METHODS

Test Patients

Fifty-three patients testing positive for serum anti-B19 IgM between 1987 and 1992 were studied. The definition of a case of B19 infection was a positive test for serum anti-B19 IgM. Each of these 53 patients was reassessed in August, 1994; the period of follow-up ranged from 26 to 85 months, with a mean of 57 months. At this time, clinical symptoms of B19 infection were recorded and blood was taken for anti-B19 antibody testing (test sera). Of the 53 patients for whom follow-up assessment was successful, serum samples from the time of initial B19 infection were available for 33 (presentation sera).

Control Patients

A control group was included as a comparison for both the serum anti-B19 IgG and serum B19 DNA results of the test patients. Fifty-three sera from 53 age (to within 3 years)- and sex-matched control patients were tested; these were patients whose sera tested negative for serological evidence of one of a variety of viral and bacterial diseases and whose symptoms did not include rash or arthralgia (control sera).

IgM Capture Radioimmunoassay

From January, 1987, to June, 1989, serum anti-B19 IgM was routinely detected in serum at initial B19 infection at the Regional Virus Laboratory by IgM captive radioimmunoassay (MACRIA), provided by Dr. Bernard Cohen, Central Public Health Laboratory, Colindale, London [Cohen et al., 1983].

IgM Capture Enzyme Immunoassay

From July 1989 to 1992, serum anti-B19 IgM was routinely detected in serum at initial B19 infection by IgM capture enzyme immunoassay (MACEIA) [O'Neill and Coyle, 1992]. To carry out the test, white, opaque, flat-bottomed Removastrips (Dynatech), used as the solid phase, were coated with a 1:1,000 dilution of rabbit anti-human IgM (Dako, High Wycombe, Bucks, England) in phosphate-buffered saline (PBS) with 0.05% Tween 20 (PBST) overnight at 4°C. Serum samples were diluted 1:100 in PBST with 1% skim milk (PBSTM), and 100 μl of each sample was added to appropriate wells. Three negative and three positive controls were included in each batch of tests. The test was incubated at 37°C for 90 min in a shaker/incubator (Amerlite Diagnostics, Ltd., Amersham, England). After washing, 100 μl of B19 antigen (final dilution 1:100)/monoclonal antibody R92F6 (final dilution 1:200) complex was added to each well, and the strips were again shake/incubated at 37°C for 90 min. After washing, 100 μl of goat anti-mouse horseradish peroxidase (HRP) conjugate (Bio-Rad) was added at its working dilution of 1:3,000. After incubation and a final wash, signal reagent (Amersham) was added. The basis of the signal reagent was luminol, which is oxidised in the presence of hydrogen peroxide and horseradish peroxidase (HRP); chemiluminescence occurs when the energy from this reaction is emitted in the form of light. The test was read 10 min after addition of signal reagent using a chemiluminescent reader (Amerlite Analyser, Amerlite Diagnostics, Amersham, England).

Preparation of Recombinant B19 Capsid Proteins for Immunofluorescence

Recombinant *Autographica california* nuclear polyhedrosis virus expressing B19 VP1 capsid protein (AcNPVB19VP1) was grown in SF9 cells derived from *Spodoptera frugiperda,* the fall army worm, and maintained at 28°C in supplemented TC100 medium (Gibco/BRL). Recombinant virus-infected cells showing a high degree of expression of B19 capsid protein were removed from the monolayer using glass beads, centrifuged, and resuspended in PBS, to give 1×10^7 cells/ml. This suspension was spotted onto immunofluorescence slides to give a uniform cell monolayer [Kerr et al., 1995a].

Serum Anti-B19 Fluorescent Antibody Test

For IgG determination, 25 μl of a 1:50 dilution of each serum was added to immunofluorescence slide wells and incubated for 30 min at 37°C in a humidified chamber. After washing in PBS, 25 μl of goat anti-human IgG fluorescein isothiocyanate (FITC) conjugate (Sigma) at its working dilution of 1:30 in PBS was applied for 30 min at 37°C. After a further wash in PBS, the slides were mounted and viewed using a fluorescence microscope (Zeiss). For IgM determination, serum was diluted 1:10 in GullSORB (Gull Laboratories, Utah, USA) and allowed to stand at room temperature for 15 min, to precipitate competing specific IgG and rheumatoid factor (RF); 20 μl of this was then added to immunofluorescence slide wells. From this point, the test was conducted as described above, except that incubations were for 1 hr and the conjugate used was goat anti-human IgM FITC (Sigma) at its working dilution of 1:50 in PBS. For each test run, known negative and positive serum controls were included.

Peptide Synthesis

To prepare a nitrocellulose strip containing epitopes present on the unique region of B19 VP1, 11 sequential, overlapping peptides, spanning the entire 226 amino acid unique portion of VP1 were synthesised. Each peptide was 38 amino acids long and overlapped the adjacent peptide by 19 amino acids. Peptides were synthesised on TentraGel S-RAM resin (Rapp Polymere, Tubingen, Germany), a polystyrene-polyoxethylene graft copolymer functionalised with the acid-labile modified Rink linker in order to generate carboxy-ter-

Peptide 1	NH_2 - MSKKSGKWWESDDKFAKAVYQQFVEFYEKVTGTDLELI - COOH
Peptide 2	NH_2 - YQQFVEFYEKVTGTDLELIQILKDHYNISLDNPLENPS - COOH
Peptide 3	NH_2 - QILKDHYNISLDNPLENPSSLFDLVARIKNNLKNSPDL - COOH
Peptide 4	NH_2 - SLFDLVARIKNNLKNSPDLYSHHFQSHGQLSDHPHALS - COOH
Peptide 5	NH_2 - YSHHFQSHGQLSDHPHALSSSSSHAEPRGENAVLSSED - COOH
Peptide 6	NH_2 - SSSSHAEPRGENAVLSSEDLHKPGQVSVQLPGTNYVGP - COOH
Peptide 7	NH_2 - LHKPGQVSVQLPGTNYVGPGNELQAGPPQSAVDSAARI - COOH
Peptide 8	NH_2 - GNELQAGPPQSAVDSAARIHDFRYSQLAKLGINPYTHW - COOH
Peptide 9	NH_2 - HDFRYSQLAKLGINPYTHWTVADEELLKNIKNETGFQA - COOH
Peptide 10	NH_2 - TVADEELLKNIKNETGFQAQVVKDYFTLKGAAAPVAHF - COOH
Peptide 11	NH_2 - QVVKDYFTLKGAAAPVAHFQGSLPEVPAYNASEKYPSM - COOH

Fig. 1. Amino acid sequence of peptides spanning the unique region of B19 VP1. A, Alanine; L, Leucine; R, Arginine; K, Lysine; N, Asparagine; M, Methionine; D, Aspartic acid; F, Phenylalanine; C, Cysteine; P, Proline; Q, Glutamine; S, Serine; E, Glutamic acid; T, Threonine; G, Glycine; W, Tryptophan; H, Histidine; Y, Tyrosine; I, Isoleucine; V, Valine.

minal amides upon cleavage. Fmoc-α-amino group protection and t-butyl-based side chain protection were employed. The guanidino group of arginine was protected by a 2,2,5,7,8-pentamethylchroman-6-sulphonyl moiety. The imidazole group of histidine was protected with either t-boc or trityl, and the sulfhydryl group of cysteine was protected with a trityl group. Couplings were carried out using either preformed O-pentafluorophenyl esters or by activation of free acids using one equivalent of O-(1H-benzotriazol-1-yl)-N,N,N′,N′-tetra-methyluronium tetrafluoroborate, one equivalent of 1-hydroxybenzotriazole, and 2 equivalents of N-methylmorpholine. Synthesis was carried out on a Milligen 9050 PepSynthesizer (Millipore, Bedford, MA) using continuous flow procedures. Following cleavage with trifluoroacetic acid in the presence of scavengers and extraction with diethylether, peptides were analysed by C^{18}-reverse-phase chromatography. After synthesis the peptides were transferred to nitrocellulose and cut into strips, each strip containing each of the 11 peptides for use as an immunoblot. Peptide 1 occurs at the amino-terminal of the unique B19 VP1 region, and peptide 11 occurs at the carboxy-terminal of the unique B19 VP1 region [Shade et al., 1986]. The sequences of peptides 1–11 are shown in Figure 1.

Immunoblot for Antibodies to the Unique B19 VP1 Region

Immunoblot assay was used to detect IgG to epitopes present on the unique region of B19 VP1 in presentation, test, and control sera. Strips were immersed in a 1% PBSTM solution and incubated for 30 min at 37°C to block free protein binding sites. Test and control sera were diluted 1:50 in 1% PBSTM, and 1 ml of each serum dilution was added to a strip in a plastic boat. Tests were then incubated for 30 min at 37°C with agitation on a spiral mixer (Spiramix, Denley). After two 5 min washes in PBST, 1 ml of a 1:3,000 solution of goat anti-human HRP conjugate (Bio-Rad) in PBSTM was added to each strip and incubated with agitation for 30 min at 37°C. Strips were then washed twice in PBST and developed with a substrate solution of 0.5 mg/ml diaminobenzidine in 50 mMol Tris HCl, pH 7.5 (DAB).

Statistical Analysis

McNemar's test was used to analyse the significance of serum anti-B19 IgG in test compared with control patients. Yates-corrected χ^2 test was used to analyse the significance of antibodies to the unique region of B19 VP1 in patients with a short duration of symptoms (<1 month) compared to those with those with a long duration of symptoms (>1 month). Yates-corrected, Fisher's exact two-tailed test was used to analyse the significance of antibodies to the unique region of B19 VP1 in B19-infected patients for whom there was a short interval between onset and blood sampling (<15 days) compared to patients for whom there was a long interval between onset and blood sampling (>15 days).

RESULTS
Clinical Aspects

Table I shows the clinical details of B19 infection among the 53 patients. Of these patients, the female to male ratio was 7.5:1. The patients' ages ranged from 5 to 49 years, with a mean of 31 years. Twelve patients were managed in hospital, and 41 patients were managed in the community. The period of follow-up ranged from 26 to 85 months, with a mean of 57 months. Clinical information was as follows: symptoms of acute B19 infection, joints affected by arthralgia if present, duration of symptoms from the time of acute B19 infection, all symptoms present during the follow-up period, and joints affected by arthralgia if present during the follow-up period.

Symptoms at the time of acute B19 infection were rash and arthralgia (n = 26), rash (n = 7), arthralgia (n = 16), TAC (n = 3), and intrauterine death (n = 1). One patient with rash and arthralgia had a history of hereditary spherocytosis but did not suffer TAC. One and two patients with arthralgia also had lymphadenopathy and localised muscle weakness, respectively. There were 42 patients with arthralgia in total. Particular joints affected were the fingers, wrists, elbows, shoulders, hips, knees, and ankles. Among patients with arthralgia (n = 42), the knee was affected in 39 cases. In 24 cases it was the only joint affected. Five patients had generalised arthralgia, involving more than five of the above-mentioned joints. In all cases of acute B19 infection, the arthralgia affected joints in a symmetrical pattern.

At follow-up assessment, the total duration of clinical symptoms after the initial acute B19 infection was determined. Among the seven patients whose acute symptoms consisted of rash alone, all showed disease resolution within 1 month; none had relapsed by the time of follow-up. Among the 42 patients with arthralgia (with or without rash), 19 cases had symptoms that resolved within 1 month, and the remainder (n = 23) had symptoms persisting longer. Among these 23 patients with persistent arthralgia, in three cases the symptoms had resolved within 1 year and in 20 cases symptoms persisted for 4–7 years; in 11 of them, symptoms were present constantly; in the remaining nine cases, symptoms were intermittent. The pattern of joint involvement at the time of follow-up in those patients with persistent arthralgia tended to be different from that at the time of initial presentation. However, in all patients with persistent arthralgia, arthralgia was part of the initial presentation. In two cases with persistent arthralgia for more than 1 year, a rash was also present with the arthralgia (patients 12 and 17). Patient 48, who had had persistent arthralgia for more than 1 year in her knees, elbows, and shoulders, had been diagnosed with rheumatoid arthritis (RA) and was attending a rheumatology clinic. This patient had no symptoms prior to B19 infection.

In two cases with persistent arthralgia, chronic fatigue was also present (patients 27 and 51); this was marked in both cases. In patient 51, a 22-year-old woman at university (at follow-up), the clinical impact of this was that she could no longer play energetically at sports (due to both general tiredness and pain in her knee and shoulder), stay up late at night, or study for more than 1 hour at a time (due to poor concentration); this was a significant change for her in that she had had remarkable energy prior to acute B19 infection in March, 1989. In patient 27, a 49-year-old professional man (at follow-up), longstanding extreme tiredness necessitated early retirement and loss of a successful business. This man subsequently became clinically depressed.

Three patients had TAC at acute B19 infection. Of these, two were asymptomatic at follow-up and one (patient 50) had a chronic undiagnosed red cell dysplasia, which necessitated occasional blood transfusion.

Serum Anti-B19 Antibody Testing

Among the test patients, in 32 cases the interval between symptom onset at presentation and the date of blood sampling was known; this ranged from 1 to 60 days, with a mean of 13.5 days. All 53 patients were serum anti-B19 IgM (MACRIA or MACEIA) positive at initial B19 infection (the sole criterion used to diagnose acute B19 infection). Among 33 presentation sera, 32 were tested for anti-B19 IgG (fluorescent antibody test; FAT). In 29 of these sera this test was positive; in the three cases in which serum at presentation was anti-B19 IgG negative, the interval between onset of symptoms and blood sampling was only 1–2 days. All test and control sera were negative for anti-B19 IgM. All 53 test sera were positive for anti-B19 IgG compared to 45 of 53 control sera, a significant difference (two-tailed P value = 0.008).

Antibodies to the Unique B19 VP1 Region

In 33 cases, serum at presentation was tested for IgG antibodies to the unique region of B19 VP1 (anti-B19 unique VP1 IgG) by immunoblot. Of these sera, 16 were positive. Of the 11 peptides present on the nitrocellulose strip, a total of four were variably recognised by these sera; peptides 2, 6, 8, and 9 (Table II). In seven cases, bands 8 and 9 were detected; in seven cases, bands 2, 8, and 9 were detected; in one case band 2 was detected; and, in one case, bands 2 and 6 were detected. Among the 17 cases who were tested by this method and were negative, three had serum anti-B19 IgG antibody detected by the FAT; the four remaining cases were serum anti-B19 IgG negative by FAT. All test and control sera were negative for anti-B19 unique VP1 IgG.

Among the group of 33 patients tested for serum anti-B19 unique VP1 IgG at presentation, 18 had a short duration of symptoms (<1 month), and 15 had a longer duration of symptoms (>1 month). With regard to serum anti-B19 unique VP1 IgG detected at presentation, of the 18 who had a short duration of symptoms, eight were positive and, of the 15 who had a longer duration

TABLE I. Symptoms in 53 B19-Infected Persons

Patient no.	Symptoms at onset[a]	Joints affected at onset[b]	Duration of symptoms[c] (months)	Symptoms at follow-up[a]	Joints affected at follow-up[b]
1	R, A	FWE	<1	—	—
2	R, A	Gen	64; i	A	H (R)
3	R, A	FK	6	—	—
4	R, A	S	85; i	A	S (R)
5	AC, HS	—	<1	—	—
6	R, A	K	<1	—	—
7	R, A	K	<1	—	—
8	R, A	Gen	<1	—	—
9	R, A	Gen	64; i	A	K (R)
10	R, A	KES	60	A	KES
11	A	K	<1	—	—
12	R, A	Gen	67	R, A	Gen
13	R	—	<1	—	—
14	R	—	<1	—	—
15	R, A	KF	63; i	A	KF
16	A	K	<1	—	—
17	R, A	K	60; i	R, A	Gen
18	R, A	Gen	59	A	Gen
19	A	KAF	73; i	A	KAF
20	R, A	K	<1	—	—
21	IUD	—	—	—	—
22	A	K	47	A	KF
23	A, W	K	4	—	—
24	R	—	<1	—	—
25	A	KW	3	—	—
26	R, A	K	<1	—	—
27	A, W	FTHK	51	A, CF	FTH
28	AC, HS	—	<1	—	—
29	R, A, HS	K	<1	—	—
30	R, A	K	<1	—	—
31	R, A	KF	48; i	A	F
32	R, A	KAF	<1	—	—
33	R, A	K	<1	—	—
34	R, A	KAF	66	A	F
35	R	—	<1	—	—
36	R	—	<1	—	—
37	R, A	K	<1	—	—
38	A	K	61	A	K
39	A	K	<1	—	—
40	A	K	61	A	K
41	R	—	<1	—	—
42	A	KAF	59	A	KAF
43	R, A	K	<1	—	—
44	R, A	K	52; i	A	KWF
45	R, A	K	<1	—	—
46	R	—	<1	—	—
47	A	K	<1	—	—
48	A	K	48; i	A	KES (ra)
49	A	K	<1	—	—
50	AC	—	26	CHA	—
51	A	KS	65	A, CF	KS
52	R, A	K	<1	—	—
53	A, L	S	51	A	S (R)

[a]R, rash; A, arthralgia; AC, aplastic crisis; HS, hereditary spherocytosis; IUD, intrauterine death; W, localised muscle weakness; CHA, chronic haemolytic anaemia; L, lymphadenopathy; CF, chronic fatigue.
[b]F, fingers; W, wrists; E, elbows; K, knees; S, shoulders; A, ankles; H, hips; Gen, generalised polyarthropathy; R, right side; ra, diagnosed and treated as rheumatoid arthritis.
[c]i, Intermittent symptoms.

of symptoms, eight were positive (Table II), a nonsignificant difference ($\chi^2 = 0.03$; degrees of freedom = 1; $P = 0.87$).

Among the total of 53 patients, there were 21 for whom presentation serum was tested for anti-B19 unique VP1 IgG and for whom the interval between onset of symptoms at presentation and blood sampling was known. With regard to this interval, for 13 of 21, this was <15 days (short interval), and, for the remaining eight cases, this interval was ≥15 days (long interval). Among the 13 cases with a short interval, nine were positive for serum anti-B19 unique VP1 IgG, and,

TABLE II. Serum Anti-B19 Antibody Testing and Period of Follow-Up

Test patient no.	Onset interval[a] (days)	IgM at onset	IgG at onset	Unique B19VP1 IgG at onset	Follow-up interval (months)
1	11	+	+	8,9	66
2	—	+	NT[b]	NT	64
3	6	+	NT	NT	50
4	7	+	+	8,9	85
5	—	+	+	—	70
6	—	+	+	2,8,9	64
7	—	+	+	2,8,9	64
8	4	+	+	2,8,9	64
9	—	+	+	2,8,9	64
10	10	+	NT	NT	60
11	—	+	NT	NT	67
12	—	+	NT	NT	67
13	—	+	+	—	64
14	20	+	+	—	63
15	—	+	+	2,6	63
16	7	+	+	2,8,9	65
17	—	+	NT	—	60
18	30	+	+	8,9	59
19	4	+	NT	NT	73
20	2	+	−	—	55
21	—	+	+	—	50
22	14	+	+	8,9	47
23	24	+	+	—	61
24	5	+	NT	NT	55
25	—	+	NT	NT	53
26	—	+	NT	NT	53
27	16	+	+	—	51
28	9	+	+	—	51
29	—	+	NT	NT	50
30	5	+	NT	NT	49
31	10	+	+	8,9	48
32	—	+	+	—	47
33	16	+	NT	NT	42
34	43	+	+	—	66
35	1	+	NT	NT	29
36	2	+	+	2,8,9	63
37	3	+	NT	NT	62
38	36	+	NT	NT	61
39	60	+	+	—	61
40	—	+	+	2,8,9	61
41	6	+	+	8,9	60
42	2	+	−	—	59
43	—	+	+	2	52
44	13	+	+	8,9	52
45	—	+	NT	NT	52
46	7	+	NT	NT	50
47	20	+	NT	NT	49
48	—	+	NT	NT	48
49	1	+	−	—	29
50	—	+	+	—	26
51	—	+	NT	NT	65
52	15	+	+	—	63
53	23	+	+	—	51

[a]Interval between symptom onset and serum sampling.
[b]NT, not tested.

among the eight cases with a long interval, one was positive (Table II), a significant difference (two-tailed P value = 0.024).

DISCUSSION

In all patients with presenting symptomatology of rash alone (n = 7), the rash had resolved by the time of follow-up assessment. Of the 42 patients presenting with arthralgia (with or without rash), 20 (48%) had symptoms persisting for the entire follow-up period; in 11 of them symptoms were constant, and in the remaining nine cases symptoms were intermittent. The pattern of joint involvement at the time of follow-up in those patients with persistent arthralgia tended to be

different from that at the time of initial presentation. In all patients with persistent arthralgia, arthralgia was part of the initial presentation. In 10 of the 20 patients with persistent arthralgia following B19 infection, nonsteroidal antiinflammatory drugs had been prescribed. Unfortunately, all patients were not assessed by a physician; direct telephone contact with the patients was the main means of clinical evaluation. Therefore, the criteria of the American Rheumatism Association (ARA) for diagnosis of RA [Arnett et al., 1988] were not applied. However, one patient (patient 48), a 44-year-old woman at presentation, who subsequently had 4 years of intermittent arthralgia in her knees, elbows, shoulders, and hands, was referred to a rheumatologist for evaluation. She had morning stiffness, soft tissue swelling of more than three joints observed by a physician, rheumatic nodules, and the presence of rheumatoid factor, which was confirmed in the present study at a titre of 1,920 IU/ml serum. This patient clearly fulfilled the criteria for a diagnosis of RA. In addition, she had a C-reactive protein of 237 mg/liter and an erythrocyte sedimentation rate of 120 mm/hr at the time of evaluation. She was started on a nonsteroidal antiinflammatory drug, to which she made a satisfactory initial response. Notably, there were two cases of chronic fatigue among the 53 patients assessed, and one of them (patient 51) was infected persistently [Kerr et al., 1995b]. The impact on both their lives since acute B19 infection had been remarkable. Further work is clearly required to assess the role of B19 in chronic fatigue.

In the present study, 33 patients were tested for serum anti-B19 unique VP1 IgG at presentation of B19 disease. Eighteen of them had a duration of illness of <1 month, and of these, eight were positive and ten were negative; 15 of 33 had a duration of illness >1 month, and of these, eight were positive and seven were negative. This is in contrast to the findings of Naides et al. [1992], who, using unique VP1 peptides as antigen, detected multiple epitope recognition in the serum of persons with asymptomatic B19 infection. However, persons with acute self-limiting B19 arthropathy and chronic B19 arthropathy lacked these antibodies.

This group of 53 B19-infected patients was generated by requests for serological virus diagnosis in patients in whom symptoms were severe enough for medical consultation, a request for serological virus diagnosis, and hospital management in some cases (23%) and does not therefore represent a typical group of B19-infected persons. This is reflected in the mean age among these patients of 31 years and a high female:male ratio of 7.5:1. B19 infection most commonly occurs in children and is thought to occur with equal frequency in males and females. The high female:male ratio seen among these patients probably reflects the high incidence of arthralgia at onset of B19 infection in this group (79%); B19 arthralgia is more common in women [Woolf et al., 1989]. The severity and high incidence of disease (38%) at follow-up may be related to the severity of acute illness in this group, a finding that is in contrast to the accepted sequence of events following acute parvovirus B19 infection in immunocompetent individuals; a short incubation, viraemia, bone marrow infection, development of specific antibody, and viral clearance [Anderson et al., 1985; Potter et al., 1987]. Therefore, the B19-infected persons in the present study have unintentionally been selected for severity of symptoms and so may not be immunocompetent.

Several regions containing neutralising epitopes have been localised to linear B19 amino acid sequences, one region at the amino terminus of VP2 at amino acids 38–87 [Yoshimoto et al., 1991] and six others distributed within the carboxy-terminal half of VP2 at amino acids 253–272, 309–330, 328–344, 359–382, 449–468; and 491–515 [Sato et al., 1991; Rogers et al., 1991]. Neutralising epitopes are also found in the unique region of VP1 [Rosenfield et al., 1992]. In the present study, serum from 16 of 33 cases at presentation was positive for specific IgG to the unique region of B19 VP1 by immunoblot. With regard to specific epitopes recognised, a differing pattern was observed between patients. In general, four peptides were recognised variably during the acute phase in these patients. In all patients except one, in whom peptides 2 and 6 were recognised, the peptides recognised were combinations of 2, 8, and 9, corresponding to amino acids 20–57, 134–171, and 153–190, respectively, from the amino-terminal of VP1. These findings are reminiscent of those of Saikawa et al. [1993] in which overlapping fusion proteins spanning the B19 capsid sequence were used to inoculate rabbits, and neutralising epitopes were identified within amino acids 31–51 and 158–227 from the amino-terminal of VP1. Synthetic peptides were used to immunise and detect the immune response in rabbits, whereas in the present study synthetic peptides were used to detect the immune response to native virus infection in humans. However, despite differences in species and source of immunogen between the two studies, the same two immunogenic regions were identified within the unique region of B19 VP1.

Regarding B19 capsids composed of recombinant VP2 protein, addition of VP1 has two effects. It allows presentation of the spike to the immune system and adds its own intrinsic neutralising determinants. Antisera raised to the unique region of VP1, 226 amino acids at the amino-terminus, precipitate empty capsids and virions, indicating that the unique region is exposed on the virion surface. These antibodies also neutralise virus infectivity [Rosenfield et al., 1992]. Linear epitopes from the VP1 unique region, presented as fusion proteins or synthetic peptides, are far more efficient at eliciting a neutralising immune response than peptides from the common or VP2 protein sequence [Saikawa et al., 1993]. VP1 presentation is thus crucial in mounting a neutralising anti-B19 immune response. Therefore, from the results of the present study, it seems that IgG specific for epitopes on the unique region of B19 VP1 may have a role only in the acute phase of the

disease; they were not detected at follow-up assessment, despite the presence of serum anti-B19 IgG in all patients.

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